

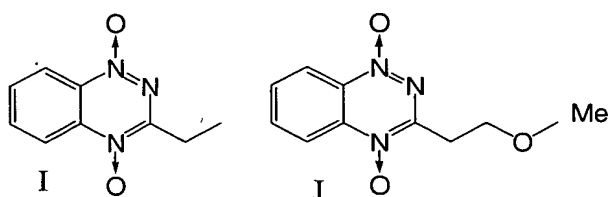
Claims

1 A method of selecting one or more 1,2,4-benzotriazine-1,4-dioxides capable
5 of in vivo hypoxia selective cytotoxicity, wherein said 1,2,4-benzotriazine-1,4-dioxide is selected if it is determined to have each of the following characteristics

- (a) a solubility greater than or about 2mM in culture medium; and
 - (b) an HT29 anoxic IC_{50} for a 4hr exposure to the 1,2,4-benzotriazine-1,4-dioxide of less than or about 40 μ M; and
 - 10 (c) a hypoxic cytotoxicity ratio (HCR) greater than about 20 for the HT29 cell line; and
 - (d) a penetration half distance (PHD) greater than or about 27 μ m, and
 - (e) the area under the plasma concentration time curve for free 1,2,4-benzotriazine-1,4-dioxide (unbound to plasma proteins), AUC_t , is greater
 - 15 than about 2 times the HT29 anoxic $IC_{50} \times t$ where $IC_{50} \times t$ is the product of concentration \times exposure time for 50% inhibition of cell proliferation
- and wherein for said 1,2,4-benzotriazine-1,4-dioxide at least one of the characteristics (a) to (e) exceeds the activity of the equivalent characteristic of Tirapazamine.

20

2 A 1,2,4-benzotriazine-1,4-dioxide having in vivo activity and selected by the method defined in claim 1, with the proviso that Tirapazamine and compounds of Formula I and J



are excluded.

25

3 A 1,2,4-benzotriazine-1,4-dioxide compound as claimed in claim 2 selected from

- N^1, N^1 -Dimethyl- N^2 -(6-methyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)-1,2-ethanediamine;
- 6-Methyl- N -[3-(4-morpholinyl)propyl]-1,2,4-benzotriazin-3-amine 1,4-dioxide;
- 30 N^1 -(6-Methoxy-1,4-dioxido-1,2,4-benzotriazin-3-yl)- N^2, N^2 -dimethyl-1,2-ethanediamine;
- N^1 -[6-(2-Methoxyethoxy)-1,4-dioxido-1,2,4-benzotriazin-3-yl]- N^2, N^2 -dimethyl-1,2-ethanediamine;

*N*¹,*N*¹-Dimethyl-*N*²-(6-ethoxy-1,4-dioxido-1,2,4-benzotriazin-3-yl)-1,2-ethanediamine;
6-Ethyl-*N*-[3-(4-morpholinyl)propyl]-1,2,4-benzotriazin-3-amine 1,4-dioxide;
2-[(3-Ethyl-1,4-dioxido-1,2,4-benzotriazin-6-yl)oxy]-*N,N*-dimethylethaneamine;
3-Ethyl-6-[3-(4-morpholinyl)propoxy]-1,2,4-benzotriazine 1,4-dioxide;
5 6-Methyl-1,2,4-benzotriazin-3-amine 1,4-dioxide; and
their pharmacologically acceptable salts thereof.

4 A method of therapy for treating cancer including the step of administering a
1,2,4-benzotriazine-1,4-dioxide compound as claimed in claim 2 or claim 3 in a
10 therapeutically effective amount to tumour cells in a subject.

5 The method as claimed in claim 4 wherein the tumour cells are in a hypoxic
environment.

15 6 The method as claimed in claim 4 or claim 5 further including the step of
administering radiotherapy to the tumour cells before, during or after the
administration of the 1,2,4-benzotriazine-1,4-dioxide compound as defined in claim 2
or claim 3 to the tumour cells.

20 7 The method as claimed in claim 6 further including the step of administering
one or more chemotherapeutic agents to the tumour cells before, during or after the
administration of the 1,2,4-benzotriazine-1,4-dioxide compound as defined in claim 2
or claim 3 to the tumour cells.

25 8 The method as claimed in claim 7 wherein the one or more chemotherapeutic
agents is selected from Cisplatin or other platinum-based derivatives, Temozolomide
or other DNA methylating agents, cyclophosphamide or other DNA alkylating agents,
Doxorubicin, mitoxantrone, camptothecin or other topoisomerase inhibitors,
Methotrexate, gemcitabine or other antimetabolites and/or Docetaxel or other
30 taxanes.

9 A method of radiosensitising in a subject tumour cells of solid tumours in
hypoxic conditions in vivo, comprising the steps of:
(a) administering to the subject a pharmaceutical composition in an amount sufficient
35 to produce radiosensitivity in the tumour cells, the composition comprising a 1,2,4-
benzotriazine-1,4 dioxide obtained by the method defined in claim 1; and

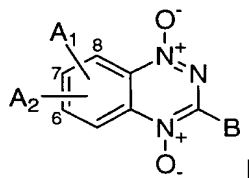
(b) subjecting the tumour cells to radiation.

10 The use in the manufacture of a medicament of a therapeutically effective
amount of a 1,2,4-benzotriazine-1,4-dioxide compound as defined in claim 2 or claim
5 3 for the treatment of tumour cells in a subject.

11 The use as claimed in claim 10 wherein the tumour cells are in a hypoxic
environment.

10 12 A pharmaceutical composition including a therapeutically effective amount of
a 1,2,4-benzotriazine-1,4-dioxide as defined in claim 2 or claim 3 and a
pharmaceutically acceptable excipient, adjuvant, carrier, buffer or stabiliser.

13 A 1,2,4-benzotriazine-1,4-dioxide compound of Formula I
15



wherein

A₁ or A₂ represent independently an H or R substituent at positions 6, 7 or 8 and/or
an OR substituent at positions 6 or 8

20 wherein each R independently represents a C₁₋₄ alkyl or cyclic C₃₋₈ alkyl
optionally substituted with substituents selected from OH, OMe, or NR¹R¹ and
wherein each R¹ is independently selected from H or a C₁₋₃ alkyl or the R¹R¹
substituents together form a morpholine ring;

B represents NHR² or R³;

25 wherein R² is a C₁₋₃ alkyl optionally substituted with substituents selected from
OH, OMe, or NR⁴R⁴

wherein R³ is selected from a C₁₋₃ alkyl optionally substituted with OH, OMe,
wherein each R⁴ is independently selected from H, a C₁₋₃ alkyl,
optionally substituted with OMe, or R⁴R⁴ together form morpholine;

30 or a pharmacologically acceptable salt thereof, and;

having the characteristics

(a) a solubility greater than or about 2mM in culture medium; and

- (b) an HT29 anoxic IC_{50} for a 4hr exposure to the 1,2,4-benzotriazine-1,4-dioxide of less than or about 40 μM ;
- (c) a hypoxic cytotoxicity ratio (HCR) greater than about 20 for the HT29 cell line; and
- 5 (d) a penetration half distance (PHD) greater than or about 27 μm , and
- (e) the area under the plasma concentration time curve for free 1,2,4-benzotriazine-1,4-dioxide (unbound to plasma proteins), AUC_t , is greater than about 2 times the HT29 anoxic $IC_{50} \times t$ where $IC_{50} \times t$ is the product of concentration \times exposure time for 50% inhibition of cell proliferation
- 10 and wherein for said 1,2,4-benzotriazine-1,4-dioxide at least one of the characteristics (a) to (e) exceeds the activity of the equivalent characteristic of Tirapazamine; and
- with the proviso that A_1 and A_2 do not both represent H when B represents CH_2CH_3 or $CH_2CH_2OCH_3$; and
- 15 with the further proviso that when A_1 represents H and A_2 represents 7-Me then B cannot represent $NH(CH_2)_2NMe_2$.

- 14 A 1,2,4-benzotriazine-1,4-dioxide compound of Formula I as claimed in claim 13 selected from
- 20 N^1, N^1 -Dimethyl- N^2 -(6-methyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)-1,2-ethanediamine;
 - 6-Methyl- N -[3-(4-morpholinyl)propyl]-1,2,4-benzotriazin-3-amine 1,4-dioxide;
 - N^1 -(6-Methoxy-1,4-dioxido-1,2,4-benzotriazin-3-yl)- N^2, N^2 -dimethyl-1,2-ethanediamine;
 - N^1 -[6-(2-Methoxyethoxy)-1,4-dioxido-1,2,4-benzotriazin-3-yl]- N^2, N^2 -dimethyl-1,2-
 - 25 ethanediamine;
 - N^1, N^1 -Dimethyl- N^2 -(6-ethoxy-1,4-dioxido-1,2,4-benzotriazin-3-yl)-1,2-ethanediamine;
 - 6-Ethyl- N -[3-(4-morpholinyl)propyl]-1,2,4-benzotriazin-3-amine 1,4-dioxide;
 - 2-[(3-Ethyl-1,4-dioxido-1,2,4-benzotriazin-6-yl)oxy]- N, N -dimethylethaneamine;
 - 3-Ethyl-6-[3-(4-morpholinyl)propoxy]-1,2,4-benzotriazine 1,4-dioxide;
 - 30 6-Methyl-1,2,4-benzotriazin-3-amine 1,4-dioxide; and
 - their pharmacologically acceptable salts thereof.

- 15 A method of therapy for treating cancer including the step of administering a 1,2,4-benzotriazine-1,4-dioxide compound of Formula I as claimed in claim 13 or
- 35 claim 14 in a therapeutically effective amount to tumour cells in a subject.

16 The method as claimed in claim 15 wherein the tumour cells are in a hypoxic environment.

17 The method as claimed in claim 15 or claim 16 further including the step of
5 administering radiotherapy to the tumour cells before, during or after the administration of the 1,2,4-benzotriazine-1,4-dioxide compound as defined in claim 13 or claim 14 to the tumour cells.

18 The method as claimed in claim 17 further including the step of administering
10 one or more chemotherapeutic agents to the tumour cells before, during or after the administration of the 1,2,4-benzotriazine-1,4-dioxide compound of Formula I as defined in claim 13 or claim 14 to the tumour cells.

19 The method as claimed in claim 18 wherein the one or more
15 chemotherapeutic agents is selected from Cisplatin or other platinum-based derivatives, Temozolomide or other DNA methylating agents, cyclophosphamide or other DNA alkylating agents, Doxorubicin, mitoxantrone, camptothecin or other topoisomerase inhibitors, Methotrexate, gemcitabine or other antimetabolites and/or Docetaxel or other taxanes.

20

20 A method of radiosensitising in a subject tumour cells of solid tumours in hypoxic conditions in vivo, comprising the steps of:

(a) administering to the subject a pharmaceutical composition in an amount sufficient to produce radiosensitivity in the tumour cells, the composition comprising a 1,2,4-

25 benzotriazine-1,4 dioxide as claimed in claim 13 or claim 14; and

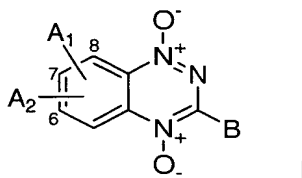
(b) subjecting the tumour cells to radiation.

21 The use in the manufacture of a medicament of a therapeutically effective amount of a 1,2,4-benzotriazine-1,4-dioxide compound of Formula I as claimed in
30 any claim 13 or claim 14 for the treatment of tumour cells in a subject.

22 The use as claimed in claim 21 wherein the tumour cells are in a hypoxic environment.

23 A pharmaceutical composition including a therapeutically effective amount of a 1,2,4-benzotriazine-1,4-dioxide of Formula I as defined in claim 13 or claim 14 and a pharmaceutically acceptable excipient, adjuvant, carrier, buffer or stabiliser.

5 24 A compound of Formula I or a pharmacologically acceptable salt thereof,



wherein

A₁ or A₂ represent independently an H or R substituent at positions 6, 7 or 8 and/or an OR substituent at positions 6 or 8

10 wherein each R independently represents a C₁₋₄ alkyl or cyclic C₃₋₈ alkyl optionally substituted with substituents selected from OH, OMe, or NR¹R¹ and wherein each R¹ is independently selected from H or a C₁₋₃ alkyl or the R¹R¹ substituents together form a morpholine ring;

B represents NHR² or R³;

15 wherein R² is a C₁₋₃ alkyl optionally substituted with substituents selected from OH, OMe, or NR⁴R⁴

wherein R³ is selected from a C₁₋₃ alkyl optionally substituted with OH, OMe, wherein each R⁴ is independently selected from H, a C₁₋₃ alkyl, optionally substituted with OMe, or R⁴R⁴ together form a morpholine

20 ring;

or a pharmacologically acceptable salt thereof, and

with the proviso that A₁ and A₂ do not both represent H when B represents CH₂CH₃ or CH₂CH₂OCH₃; and

with the further proviso that when A₁ represents H and A₂ represents 7-Me then B

25 cannot represent NH(CH₂)₂NMe₂.

25 A compound of Formula I as claimed in claim 24 wherein A₁ represents Me, Et, OMe, OEt, or OCH₂CH₂OMe; A₂ represents H and B represents Me, Et, CH₂CH₂OH, CH₂CH₂OMe, NHCH₂CH₂NMe₂, NHCH₂CH₂Nmorpholine, or

30 NHCH₂CH₂CH₂Nmorpholine.

26 A compound of Formula I as defined in claim 24 or claim 25 wherein A₁ represents CH₂CH₂NMe₂, CH₂CH₂Nmorpholine, CH₂CH₂CH₂Nmorpholine,

OCH₂CH₂NMe₂, OCH₂CH₂Nmorpholine or OCH₂CH₂CH₂Nmorpholine and B represents Me, Et, CH₂CH₂OH or CH₂CH₂OMe.

- 27 A 1,2,4-benzotriazine-1,4-dioxide compound of Formula I as claimed in claim
5 24 selected from
N¹,N¹-Dimethyl-N²-(6-methyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)-1,2-ethanediamine;
6-Methyl-N-[3-(4-morpholinyl)propyl]-1,2,4-benzotriazin-3-amine 1,4-dioxide;
N¹-(6-Methoxy-1,4-dioxido-1,2,4-benzotriazin-3-yl)-N²,N²-dimethyl-1,2-ethanediamine;
10 N¹-[6-(2-Methoxyethoxy)-1,4-dioxido-1,2,4-benzotriazin-3-yl]-N²,N²-dimethyl-1,2-ethanediamine;
N¹,N¹-Dimethyl-N²-(6-ethoxy-1,4-dioxido-1,2,4-benzotriazin-3-yl)-1,2-ethanediamine;
6-Ethyl-N-[3-(4-morpholinyl)propyl]-1,2,4-benzotriazin-3-amine 1,4-dioxide;
2-[(3-Ethyl-1,4-dioxido-1,2,4-benzotriazin-6-yl)oxy]-N,N-dimethylethaneamine;
15 3-Ethyl-6-[3-(4-morpholinyl)propoxy]-1,2,4-benzotriazine 1,4-dioxide;
6-Methyl-1,2,4-benzotriazin-3-amine 1,4-dioxide; and
their pharmacologically acceptable salts thereof.

- 28 A method of therapy for treating cancer including the step of administering a
20 1,2,4-benzotriazine-1,4-dioxide compound of Formula I as claimed in any one of claims 24 to 27 in a therapeutically effective amount to tumour cells in a subject.

- 29 The method as claimed in claim 28 wherein the tumour cells are in a hypoxic environment.

25

- 30 The method as claimed in claim 28 or claim 29 further including the step of administering radiotherapy to the tumour cells before, during or after the administration of the 1,2,4-benzotriazine-1,4-dioxide compound of Formula I as defined in any one of claims 24 to 27 to the tumour cells.

30

- 31 The method as claimed in claim 30 further including the step of administering one or more chemotherapeutic agents to the tumour cells before, during or after the administration of the 1,2,4-benzotriazine-1,4-dioxide compound of Formula I as defined in any one of claims 24 to 27 to the tumour cells.

35

32 The method as claimed in claim 31 wherein the one or more
chemotherapeutic agents is selected from Cisplatin or other platinum-based
derivatives, Temozolomide or other DNA methylating agents, cyclophosphamide or
other DNA alkylating agents, Doxorubicin, mitoxantrone, camptothecin or other
5 topoisomerase inhibitors, Methotrexate, gemcitabine or other antimetabolites and/or
Docetaxel or other taxanes.

33 A method of radiosensitising in a subject tumour cells of solid tumours in
hypoxic conditions in vivo, comprising the steps of:
10 (a) administering to the subject a pharmaceutical composition in an amount sufficient
to produce radiosensitivity in the tumour cells, the composition comprising a 1,2,4-
benzotriazine-1,4 dioxide as claimed in any one of claims 24 to 27; and
(b) subjecting the tumour cells to radiation.

15 34 The use in the manufacture of a medicament of a therapeutically effective
amount of a 1,2,4-benzotriazine-1,4-dioxide compound of Formula I as defined in any
one of claims 24 to 27 for the treatment of tumour cells in a subject.

35 The use as claimed in claim 34 wherein the tumour cells are in a hypoxic
20 environment.

36 A pharmaceutical composition including a therapeutically effective amount of
a 1,2,4-benzotriazine-1,4-dioxide of Formula I as defined in any one of claims 24 to
27 and a pharmaceutically acceptable excipient, adjuvant, carrier, buffer or stabiliser